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# Behavioral Problems After Early Life Stress: Contributions of the Hippocampus and Amygdala

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## ABSTRACT

**BACKGROUND:** Early life stress (ELS) can compromise development, with higher amounts of adversity linked to behavioral problems. To understand this linkage, a growing body of research has examined two brain regions involved with socioemotional functioning—amygdala and hippocampus. Yet empirical studies have reported increases, decreases, and no differences within human and nonhuman animal samples exposed to different forms of ELS. This divergence in findings may stem from methodological factors, nonlinear effects of ELS, or both.

**METHODS:** We completed rigorous hand-tracing of the amygdala and hippocampus in three samples of children who experienced different forms of ELS (i.e., physical abuse, early neglect, or low socioeconomic status). Interviews were also conducted with children and their parents or guardians to collect data about cumulative life stress. The same data were also collected in a fourth sample of comparison children who had not experienced any of these forms of ELS.

**RESULTS:** Smaller amygdala volumes were found for children exposed to these different forms of ELS. Smaller hippocampal volumes were also noted for children who were physically abused or from low socioeconomic status households. Smaller amygdala and hippocampal volumes were also associated with greater cumulative stress exposure and behavioral problems. Hippocampal volumes partially mediated the relationship between ELS and greater behavioral problems.

**CONCLUSIONS:** This study suggests ELS may shape the development of brain areas involved with emotion processing and regulation in similar ways. Differences in the amygdala and hippocampus may be a shared diathesis for later negative outcomes related to ELS.

*Keywords:* Abuse, Amygdala, Chronic stress, Development, Early life stress, Emotion, Hippocampus, Limbic system, Medial temporal lobe, Neglect, Neural plasticity, Neuroimaging, Poverty, Stress

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It is increasingly clear that early life stress (ELS) can compromise development, with research linking experiences such as child maltreatment or chronic poverty with behavioral problems, such as aggressive and oppositional behavior (1). Such problems are associated with substantial financial costs and sow the seeds for later psychopathology (2–4). To make inroads in conceptualizing, studying, and treating these problem behaviors, more recent work has focused on neurobiological risks (5–8). However, this research has not strongly focused on ELS. This gap is a major limitation because these behaviors often emerge after exposure to varying forms of ELS (9–25). To date, there have been very few investigations on the neurobiology of ELS and behavioral problems. These limited investigations have focused on brain regions involved in emotion processing and regulation, such as the prefrontal cortex (PFC), hippocampus, and amygdala (26). Consensus has begun to materialize regarding ELS and the PFC, with many studies reporting differences in this brain region after ELS (27,28). However, similar agreement does not exist for the hippocampus and amygdala, with inconsistent results being reported even in meta-analyses on the neurobiological effects of trauma (29,30). Resolving these inconsistencies is essential to understanding neural alterations associated with ELS and behavioral problems.

Divergence in these findings is not surprising when one considers that past human studies of ELS often relied on "natural experiments" focused on samples exposed to stressful experiences. These retrospective designs, although informative, have many significant limitations including the lack of random assignment. Working with multiple groups of children exposed to different forms of adversity is one fruitful way to overcome these limitations and has important advantages over past studies. First, limitations related to unobserved or unmeasured characteristics of specific stressful experiences can be minimized. For example, physical abuse is associated with familial poverty throughout development, more so than

 
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early neglect during institutionalization (31,32). Finding brain differences in both samples may indicate common neurobiological diatheses. Second, the timing, chronicity, and scope of stress may differ greatly between groups; however, the behavioral end-state (behavioral problems) is similar across populations. For example, children who experience early neglect commonly experience unresponsive caregiving and an overall dearth of individualized care and attention (33). In contrast, children who have been victims of physical abuse may interact with parents often, but these experiences may involve excessive physical aggression directed at the children (34). Examining different groups exposed to different forms of ELS is a powerful way to understand whether similar or unique patterns of neurobiological alterations put individuals at risk for behavioral problems.

Past research implicates the amygdala and hippocampus in basic socioemotional functioning, making them candidate brain regions for understanding behavioral problems following ELS. The hippocampus is involved in learning, memory, and the neuroendocrine response to stress (35,36). The amygdala is central to emotional and social information processing, with damage to this area leading to problems in evaluating the significance of social stimuli (37,38). However, major inconsistencies have emerged in research examining these structures in human and nonhuman samples exposed to stress (39).

Chronic stress causes reductions in dendritic spines and apoptosis of hippocampal neurons in adult nonhuman animals (40–42). In humans, one form of ELS, child maltreatment, is consistently related to smaller hippocampi in adults (30,43,44). Earlier in development while the hippocampus is still changing, these findings are less clear. Smaller hippocampi have been reported in children living in poverty (45–47) and children exposed to ELS such as parental separation or loss (48). However, no differences in hippocampi have been found in nonhuman primates separated from their parents (49), human children exposed to early neglect and later adopted into enriched environments (50–53), or human children who experienced abuse before being diagnosed with posttraumatic stress disorder (54–57).

For the amygdala, volumetric increases such as dendritic arborization in amygdala nuclei have been reported in adult rodents exposed to stress (58–61). However, structural neuroimaging studies examining amygdala volumes in humans have been inconclusive. In children exposed to early neglect, research reports have noted larger amygdalae (50,51) as well as no differences (52,53). Child poverty has been associated with larger (46) as well as smaller (47) amygdalae. Smaller amygdalae (62) as well as no differences (54–57) have been found in adolescents who experienced child maltreatment. Many previous investigations in humans (45,46,51,55,56) have had a large age range of participants (e.g., 5–15 years old); this is particularly important to note because amygdala development appears to be nonlinear in nature (63,64).

Divergence in results may also be due to methodological factors, such as magnetic resonance imaging (MRI) acquisition parameters or amygdala and hippocampal quantification procedures (65). For example, a review of amygdala quantification found the range of volumes was 1050–3880 $mm^3$, suggesting great variance in how researchers label these regions (66). Automated quantification of the hippocampus and amygdala also may be adding to inconsistencies in research findings. Methods such as FreeSurfer yield high variability and low validity for regions such as the amygdala (67,68), often changing study results (Supplement 1) (69). To resolve prior discrepancies, highly valid and reliable measures of the amygdala and hippocampus are needed across different groups exposed to different forms of ELS.

In addition to methodological factors, the effects of stress on the medial temporal lobe (MTL) may be nonlinear with different types of volumetric alterations depending on the timing and chronicity of stress (70–72). Understanding of the effects of ELS on the MTL has been primarily informed by nonhuman animal models employing chronic immobilization stress (CIS), although other nonhuman animal paradigms exist (73). Although informative, CIS models may be hard to translate to human samples, particularly in how to understand the long-term neurobiological sequelae of ELS. For example, research suggests the amygdala may adapt and function differently after increased dendritic arborization. Enlargement of amygdala volumes (58–61) and amygdala hyperactivity (74,75) result from CIS. McEwen (76) noted parallels between these findings and patterns of brain alterations in humans during initial episodes of major depression, where larger volumes and increased functional activity of the amygdala have been noted (77,78). McEwen further suggested that this hyperactivity might give way to eventual shrinkage, citing reports of smaller amygdalae after repeated depressive episodes (79). Similar ideas have been advanced and supported in research focusing on the amygdala and autism where volumetric overgrowths have been reported early in development, but smaller volumes have been noted later in life (72,80,81). In further support of this idea, more recent work employing CIS found a single, prolonged stressor caused apoptosis of amygdala cells (82).

Based on this body of evidence, ELS may result in an initial increase in amygdala volume along with increases in activity and excitatory neurochemistry. Such speculation fits with three research reports finding higher amygdala activity in children who experienced ELS (83–85). Over time, this excessive functional activity may lead to a loss of neurons (70,74). Individuals exposed to greater amounts of stress or exhibiting greater levels of impairments may have smaller volumes caused by this hypotrophy. In regard to the hippocampus, stress is theorized to be accompanied by a glucocorticoid cascade causing smaller hippocampi over time. Initial data suggest that hippocampal alterations may "reverse" over time, with previously detected differences not present after stress-free periods. However, differences in the amygdala are seen even after stress-free periods in nonhuman animals (86). Such models help in understanding nonlinear patterns seen in other trauma-exposed populations (87) along with inconsistencies seen in previous research. For example, work by Mehta *et al.* (50) found larger amygdalae in children exposed to early neglect (a type of ELS); however, these investigators found the amount of early neglect to which these same children were exposed was actually related to smaller amygdalae.

The present study examined different forms of ELS, employing the same quantification procedures for the MTL for children who experienced early neglect, experienced physical abuse, or were from low socioeconomic status

(SES) households. This approach allowed us to examine whether similar patterns of volumetric changes might be occurring with different forms of ELS and whether this may be a shared diathesis for behavioral problems. To gain a greater understanding of how ELS might affect the brain and behavior, we also collected rigorous measures of cumulative stress exposure. Such data allow us to probe robustly the level of cumulative stress to which each child was exposed during development.

Based on theoretical models positing nonlinear effects of stress, we postulated that all three forms of ELS would lead to smaller volumes in the amygdala. This idea is motivated by the extant literature reviewed earlier and theoretical models of nonlinear changes in the amygdala after early increased dendritic arborization (70). In addition, we predicted that greater cumulative stress exposure would be associated with smaller amygdalae and that smaller amygdalae would be associated with more behavioral problems. Finally, we theorized that smaller amygdalae would help account for the contribution of cumulative stress exposure to individual differences in behavioral problems. We postulated similar hypotheses for the hippocampus.

## METHODS AND MATERIALS

### Subjects

T1-weighted MRI images were collected using a 3T General Electric SIGNA MRI scanner (GE Healthcare, Waukeshau, Wisconsin) (additional information in Supplement 1) for 128 children (61 girls; mean age, 141.9 months; SD ± 20.45; range, 108.23–178.70 months). These children constituted three different ELS risk groups: children who experienced early caregiving neglect while living in institutions for orphaned or abandoned children, children from low SES households, and children who were victims of physical abuse. Each group was recruited to allow for examination of different types of ELS. Similar data also were collected from comparison children not exposed to ELS. Informed consent from the parents or guardians of all children and informed assent from all child participants were obtained in compliance with the University of Wisconsin-Madison institutional review board. The institutional review board also approved all study procedures.

To understand the effects of drastic environmental change after ELS, 36 participants who were internationally adopted from institutions for orphaned or abandoned children after experiencing neglect (21 girls; mean age, 139.34 months; SD ± 20.2) were recruited for this study. These participants spent an average of 29.52 months (SD ± 16.681; range, 3–64 months; median, 33.0 months) in institutional care. These children were on average 38.08 months old (SD ± 22.69; median, 35.0 months; range, 3–92 months) when they were adopted. These children had environments that changed drastically after they were adopted into normative family settings.

To represent the effects of exposure to extremely volatile emotional caregiving, 31 participants who experienced physical abuse (11 girls; mean age, 144.13 months; SD ± 19.72) were recruited for this study. This sample was identified in one of two ways: 1) children whose parents scored at least 20 on the physical abuse subscale of the Conflict Tactics Scale Parent-Child Version (88), a measure of parental aggression toward their children, or 2) children whose parents had substantiated cases of physical abuse on record with the Dane County Department of Human Services.

To understand how pervasive environmental stress and lack of enrichment in the absence of overt parental aggression can influence the brain, 20 participants from low SES households (14 girls; mean age, 146.24 months; SD ± 20.15) were recruited. Low SES was defined using the Hollingshead two-factor index (89), with children from low SES households having parents that were unskilled employees with a high-school education or less (additional information in Supplement 1).

There were 41 participants who served as comparison children from middle-class SES households with no history of maltreatment (15 girls; mean age, 140.46 months; SD ± 21.57). Comparison children were required to have scores $<12$ on the Conflict Tactics Scale Parent-Child Version and to have a Hollingshead index score $>50$. Sample demographics are shown in Table S1 in Supplement 1.

### Pubertal Examination

To control for possible influences of puberty on the MTL, all children completed a physical examination with Tanner staging (Supplement 1) (90,91). Children from low SES households exhibited more advanced pubertal development than comparison children from middle-class SES households ($t = 3.54$, $p < .001$). No differences in pubertal maturation were noted for children exposed to early neglect ($t = .145$, $p = .885$) or who experienced physical abuse ($t = 1.39$, $p = .168$) compared with children from middle-class SES households. There were no group differences in age in months (all groups, $p < .3$). Group means and SDs are shown in Table S1 in Supplement 1.

### Amygdala and Hippocampal Volume of Interest Drawing

Volume of interest drawing of the amygdala was based on Nacewicz *et al.* (71). Hippocampal volumes of interest were traced based on the criteria detailed by Rusch *et al.* (92) and informed by relevant brain atlases (93,94). Extensive detail regarding tracing procedures and anatomical boundaries is available in Supplement 1. All tracing was carried out by raters blind to group, yielding high reliability (interrater intraclass correlation = .95 amygdala volumes and .93 hippocampal volumes) and high spatial reliability (mean intersection/union = .84 amygdala, $n = 13$, and .86 hippocampus, $n = 12$). Example tracings are shown in Supplement 1.

### Assessment of Behavioral Problems

The behavioral problems section of the Youth Life Stress Interview (YLSI) (95,96) was used to assess behavioral problems. Advanced graduate-level researchers conducted all interviews. A series of probes was administered to elicit information from children and parents regarding children's behavioral problems at school (e.g., problems with teachers, disciplinary actions related to disruptive behavior). A panel of three to six trained raters who did not interact with the family used a 5-point scale based on separate parent and child reports. Interviewers were trained on filtering out a

participant's subjective responses to probes (e.g., child's affect) during discussion with this rating team. After parent and child reports were scored individually, a consensual rating was assigned integrating information from both informants. Higher scores reflected more serious behavioral problems. For example, a score of 1.5 reflects a child who was rarely in trouble at school, whereas a score of 4 reflects a child who received frequent detentions at school and was often sent to the principal. High reliability has previously been achieved for ratings measuring functioning in different life domains derived from the YLSI (intraclass correlation = .96) (96,97).

### Assessment of Cumulative Life Stress

To assess cumulative life stress, interviewers administered the lifetime adversity section of the YLSI separately to children and their parents. This module of the interview assessed a child's exposure to severe negative life events and circumstances across his or her lifetime, excluding events within 1 year to distinguish recent life stressors. General and specific probes were employed to assess a child's exposure to particularly stressful events and circumstances (e.g., death of close family members, severe chronic illness of close family members). Semistructured follow-up questions were asked to assess the event's context (e.g., timing, duration).

An interviewer elicited objective information about the impact of stressors and provided this information to an independent rating team with no knowledge of the child's subjective state. Integrating across parent and child reports, the independent rating team (of three to six members) provided a consensual rating on a 10-point scale that reflected the overall level of cumulative life stress. This rating incorporated a detailed consideration of the context of events and the impact on an individual child's life, rather than simply reflecting the number of stressors. For example, death of a relative receives a uniform score within many stress checklist approaches, but the YLSI differentiates a death of a relative who played a major role in the child's life versus a relative with infrequent contact and little involvement with the child (98). Specific examples from our study are detailed in Supplement 1. The scores not only reflect the occurrence of particular stressors but also an objective assessment of the degree of impact of each stressor on the child (e.g., long-term consequences). This rating system has high reliability and validity (intraclass correlation = .99) (97).

## RESULTS

To examine whether specific forms of ELS were associated with amygdala or hippocampal differences, three separate

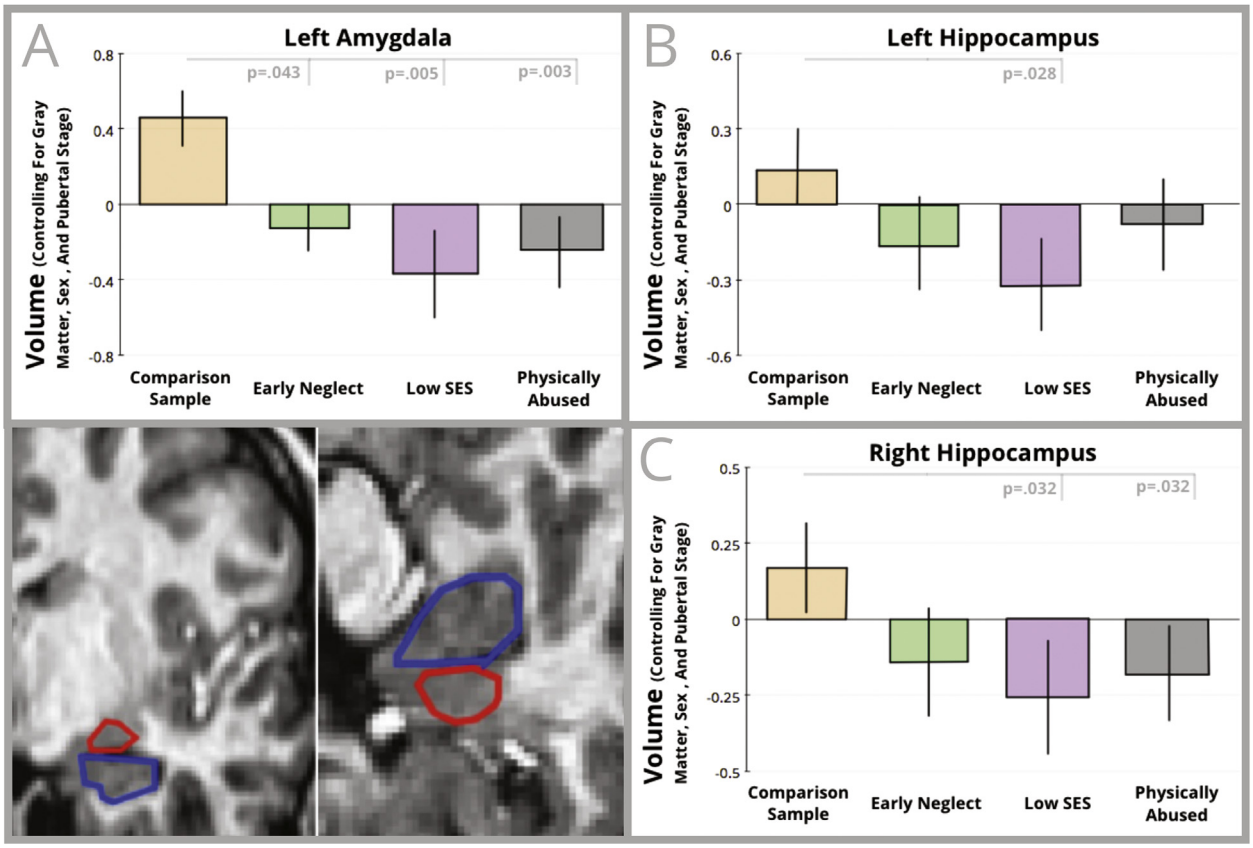


**Figure 1.** Volumetric comparisons for the left amygdala **(A)** and left **(B)** and right **(C)** hippocampus. For each graph, standardized residuals controlling for total gray matter, pubertal stage, and sex are shown on the vertical axis, and group is shown on the horizontal axis. Example hand-tracings of the amygdala (outlined in red) and hippocampus (outlined in blue) are in the bottom left corner of the figure. SES, socioeconomic status. Additional information and graphic depiction of tracings available in Supplement 1.

linear regression models were used to compare children who experienced different forms of ELS (i.e., physical abuse, early neglect, low SES) with comparison children who had not experienced ELS. Such an approach has been employed and recommended by other research groups (99,100). Right and left volumes for each structure were entered separately into linear regressions as dependent variables. Total gray matter, sex, pubertal stage, and group (dummy-coded) were entered as independent variables. In addition, SES was included as a covariate in analyses involving children who had experienced physical abuse or early neglect. Analyses controlling for age are detailed in Supplement 1.

After controlling for puberty, children who experienced early neglect ($t = -2.058$, $p = .043$) and children from low SES households ($t = -2.927$, $p = .005$) had smaller left amygdalae relative to comparison children. Smaller left ($t = -2.257$, $p = .028$) and right ($t = -2.205$, $p = .032$) hippocampi were also found for children from low SES households relative to comparison children. Children who experienced physical abuse had smaller left amygdalae ($t = -3.107$, $p = .003$) and smaller right hippocampi ($t = -2.193$, $p = .032$) relative to comparison children. These differences are shown in Figure 1.

## MTL, Cumulative Life Stress, and Behavioral Problems

Because similar patterns of volumetric differences were found in the aforementioned analyses, we collapsed across the three ELS groups and examined correlations between level of cumulative life stress and amygdala and hippocampal volumes to gain greater statistical power. For children exposed to any form of ELS, higher levels of cumulative stress were associated with smaller volumes in the left amygdala ($r = -.257$, $p = .020$) and the hippocampus (left, $r = -.229$, $p = .035$; right, $r = -.263$, $p = .015$). These relationships are shown in Figures 2 and 3. Similar associations were seen if comparison children were included in these analyses (left amygdala, $r = -.316$, $p < .001$; left hippocampus, $r = -.313$, $p < .001$; right hippocampus, $r = -.340$, $p < .001$) (Figure S3 in Supplement 1).

Next, we examined correlations between MTL volumes and behavioral problems in children exposed to ELS. Greater behavioral problems such as disobeying rules were associated with smaller left amygdala volumes ($r = -.238$, $p = .045$) and smaller hippocampal volumes (left, $r = -.271$, $p = .012$; right, $r = -.272$, $p = .012$). These associations are shown in Figures 2 and 3. Similar associations were seen if comparison children were included in analyses (left amygdala, $r = -.211$, $p = .019$; left hippocampus, $r = -.284$, $p = .001$; right hippocampus, $r = -.289$, $p = .001$) (Figure S4 in Supplement 1). Descriptive statistics on ELS and behavioral problems are presented in Supplement 1.

## MTL Mediation of ELS and Behavioral Problems

After finding these associations, we sought to investigate whether individual differences in the MTL mediated the effects of ELS on behavioral problems (using Sobel tests) (101). These tests revealed that hippocampal volumes (left hippocampus, $Z = 2.032$, $p = .042$; right hippocampus, $Z = 2.051$, SE = .013, $p = .040$) partially mediated the association between ELS and behavioral problems.[1] No such association was found for the amygdala (all $p > .22$).

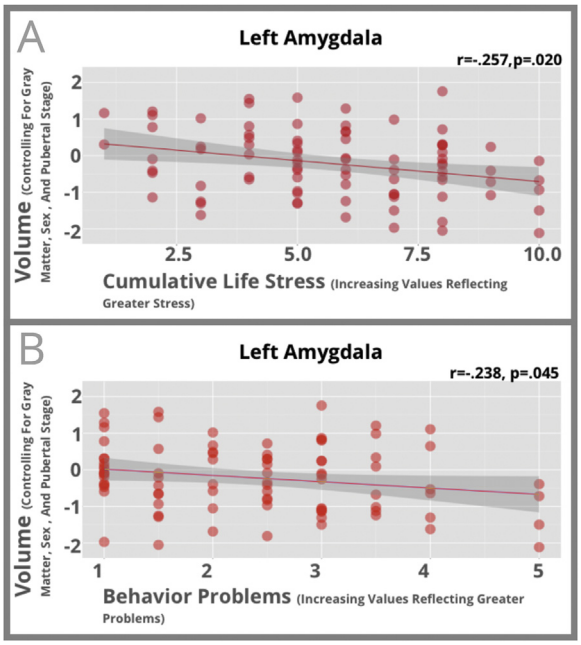


**Figure 2.** Scatterplots between left amygdala volume and cumulative stress exposure **(A)** and behavioral problems **(B)** for participants who experienced early life stress. Standardized residuals of amygdala volume controlling for total gray matter, pubertal stage, and sex are shown on the vertical axis, and cumulative stress exposure **(A)** or behavioral problems **(B)** are shown on the horizontal axis.

## DISCUSSION

The goal of this study was to understand if ELS was associated with volumetric differences in the amygdala and hippocampus, two important MTL structures involved with socioemotional functioning. By working with groups of children exposed to different forms of ELS, we additionally sought to overcome limitations of past research studies, such as unobserved or unmeasured characteristics of specific stressful experiences. Rigorous hand-tracing methods revealed that each form of ELS investigated was associated with differences in amygdala and, to some extent, hippocampal volumes. Smaller amygdalae were observed in children exposed to physical abuse, exposed to early neglect, and from low SES households compared with children who had not experienced such early adversities. In regard to the hippocampus, smaller volumes were observed in children exposed to physical abuse and children from low SES households relative to comparison children.

Our results fit with some previous findings but also stand in contrast to some of the extant literature. For the amygdala, smaller volumes in children who have experienced physical

[1]The relationship between cumulative life stress and behavioral problems was still significant when hippocampal volumes were included in regression analyses (life stress, $t = 3.7$, $p < .001$).

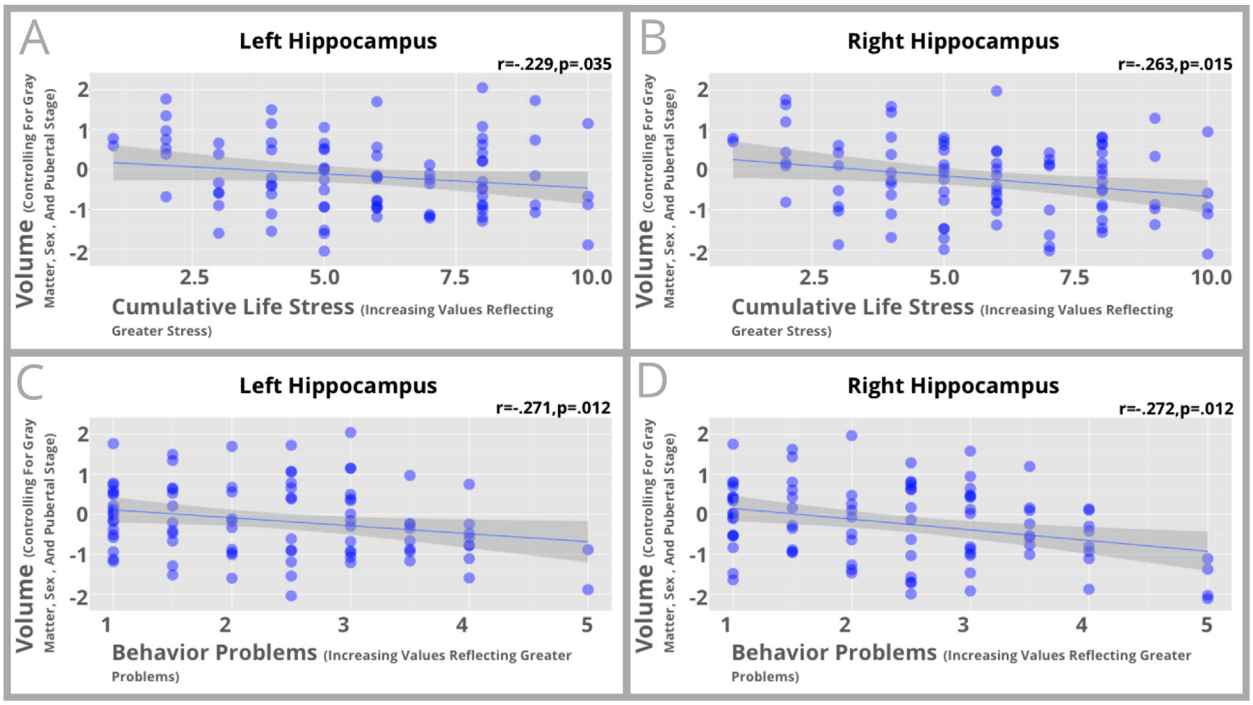


**Figure 3.** Scatterplots between hippocampal volume and cumulative stress exposure **(A, B)** and behavioral problems **(C, D)** for participants who experienced early life stress. Left hippocampus **(A, C)** and right hippocampus **(B, D)** are shown. Standardized residuals of hippocampal volume controlling for total gray matter, pubertal stage, and sex are shown on the vertical axis, and cumulative stress exposure **(A, B)** or behavioral problems **(C, D)** are shown on the horizontal axis.

abuse mirror more recent results in a similar-age sample of children who experienced this ELS (62). In regard to early neglect, our results are in contrast to previous null results and reports showing larger amygdalae in similar samples. Additionally, we found smaller amygdalae in children living in low SES households, which fits with more recent results reported by Luby *et al.* (47) but is counter to results reported by Noble *et al.* (46). Our results for the hippocampus fit well with the extant literature. In contrast to the amygdala, hippocampal alterations after stress are typically unidirectional, with smaller volumes being commonly reported. We found smaller hippocampi in children who experienced physical abuse and children from low SES households, which fits with past reports (45–47). Unique to our work, we found that greater cumulative stress exposure was associated with smaller volumes in both the amygdala and the hippocampus. Smaller volumes in these structures were associated with behavioral problems. Individual differences in hippocampal volumes partially mediated the contribution of ELS to increased levels of behavioral problems.

In considering inconsistencies in past research, it should be noted that our sample had a more narrow age range and had a larger sample size than previous reports. In regard to age range, many past studies had samples that spanned from early childhood into late adolescence [e.g., 5.22–15.76 years (51), 4.9–17.0 years (56)]. In regard to the range of ELS in this study, the amount of some forms of ELS may be higher than past work. Tottenham *et al.* (51) reported larger amygdalae in children who experienced early neglect; however, the children in that sample had experienced a shorter period of caregiving neglect than our participants (placement in institution at 2.7 months of age on average with average age of adoption of 18.8 months). Differences in institutional duration is one possible explanation why larger volumes were previously noted (51). In an older sample of children who experienced early neglect with periods of deprivation similar to our sample, Mehta *et al.* (50) reported results similar to ours. These investigators found a negative correlation with time spent in institutions, with children experiencing longer periods of neglect having smaller amygdalae. Additionally, the use of less rigorous quantification methods in previous research may partly be driving inconsistencies in the past literature. For example, as noted in Supplement 1, all associations with the amygdala are nonsignificant when employing automated segmentation methods.

Thinking broadly, we believe our results for the amygdala fit into a nonlinear model of amygdala alterations after ELS. Compelling data exist that ELS is associated with volumetric increases in the amygdala (50,51,60,61) and increased amygdala activity (83–85). Preliminary data also suggest ELS is related to increased excitation and cell death (74,75,82). With greater stress or if examined later in development, reductions in volume are expected. We believe the smaller volumes across the multiple samples we examined provide indirect support for this latter idea. However, great caution must be used when inferring developmental patterns from cross-sectional studies; only longitudinal research can truly validate such a model of amygdala development after ELS. This

nonlinear model does have implications for cross-sectional studies that distinguish it from a model of amygdala hyperfunction. The integrated structural and functional alterations in the amygdala may help us understand individual differences in risk and resilience to behavioral problems (as well as different forms of psychopathology) seen after ELS.

The study design has potential limitations. Our data are based on a single MRI scan. It is possible that brain development is simply delayed in children who were subjected to high levels of cumulative life stress. Volumetric differences could "equalize" over time; this may be particularly true of the hippocampus, where research has demonstrated reversibility in volumetric differences if given a "stress-free" period (60). Related to this idea, we did not find any differences in the hippocampus for children who experienced early neglect and subsequently had an enriched (and potentially less stressful) environment after adoption. In future work, we hope to assess other structural and functional properties of the amygdala and hippocampus through the use of longitudinal functional MRI and magnetic resonance spectroscopy (102).

In conclusion, the present study demonstrates adverse early experience is associated with structural differences in the MTL. These results are particularly important because ELS has been linked with psychopathology later in life in which this brain circuit may play a central role (103,104). Overall, children who experienced ELS had volumetric alterations in the amygdala and hippocampus. Individual differences in MTL structures, particularly for the hippocampus, were associated with behavioral problems. This research also has implications for basic science, by increasing understanding of how postnatal experience shapes brain and behavioral development. Stressful experiences with different onsets, severities, and chronicities all may have a similar impact on neurobiological circuitry related to behavioral problems. Further research is needed to determine if critical and sensitive periods exist for these processes.

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